

# Karyotype of *Sabanejewia bulgarica* (Drensky, 1928) (Teleostei, Cobitidae) from the Danube Delta, Romania

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## Abstract

The karyotype of the freshwater fish *Sabanejewia bulgarica* (Drensky, 1928), from the Danube Delta, was studied by conventional Giemsa staining and the C-banding technique. The diploid chromosome number was  $2n = 50$ . The karyotype contained 2 pairs of metacentric (the first one was much larger than the second one), 6 pairs of submetacentric and 17 pairs of subtelocentric to acrocentric chromosomes. Pericentromeric blocks of heterochromatin were revealed in most of the chromosome pairs. The karyotype phenotype of *S. bulgarica* was the same as found for *S. balcanica* from Northern Carpathian Mountains.

## Keywords

C-heterochromatin, Chromosome number, cobitoid loaches, karyotype structure

## Introduction

Freshwater fishes of the genus *Sabanejewia* Vladykov, 1929 are small (max 15 cm TL), have a benthic lifestyle in river habitats and can be distinguished from all other genera of Cobitidae by a specific sexual dimorphism (males with lateral body swellings) (Nalbant 1994; Kottelat and Freyhof 2007; Šlechtová et al. 2008). Their distribution spans from northern Italy to the Aral Sea basin including tributaries of the Black Sea, Caspian Sea,

Baltic Sea, Aral Sea and Mediterranean Sea (Kottelat and Freyhof 2007). The highest taxonomic diversity was long time assumed to be in the Danube basin, but phylogenetic studies (Perdices and Doadrio 2001; Šlechtová et al. 2008; Vasil'eva et al. 2022) have shown that several phenotypes within this area are very closely related (*Sabanejewia balcanica* (Karaman, 1922) species complex), so that the exact species composition is still not known.

*Sabanejewia bulgarica* (Drensky, 1928) was originally described as a species of the genus *Cobitis* Linnaeus, 1758 occurring in the lowest Danube basin including the Danube River itself. Junior synonyms are *C. albicoloris* Chichkoff, 1932 and *C. taenia tessellatus* Pietschman, 1937 (Kottelat 2012). For some time, most authors included *S. bulgarica* in the polytypic Balkan golden loach complex, with *Sabanejewia balcanica*, as a subspecies (Nalbant 1957; Bănărescu 1964; Bănărescu et al. 1972). Vasil'eva and Vasil'ev (1988) analysed the geographical variations of characters among a number of local forms of golden loach throughout its range they showed that *bulgarica* is quite distinct from all other populations and deserves species rank (Nalbant 1994; Kottelat 1997, 2012). The main differences include the overall colour of body, character of Gambetta's zone, pigmentation and character of spots on the end of the caudal peduncle and basis of caudal fin as well as the habitat, as it is the only deep-water riverine form of *Sabanejewia*. Recently, Križek et al. (2020) based on several lines of evidence and analysis of individuals from type localities of both *S. balcanica* and *S. bulgarica sensu* Bănărescu et al. (1972) have claimed that Danubian golden loaches are genetically closer to *S. bulgarica* and *S. balcanica* itself is to be restricted to the drainage of the Vardar River.

Chromosomes of the loaches of the genus *Sabanejewia* remain poorly studied. Ráb et al. (1991) summarized three previous studies dealing with *S. larvata* (De Filippi, 1859), *S. caspia* (Eichwald, 1838) and *S. kubanica* Vasil'eva et Vasil'ev, 1988. Vasil'ev and Vasil'eva (1994, 2019) reported karyotypes of *S. caspia* and *S. aurata* (De Filippi, 1863) from other locations (Kizylach Bay of the Caspian Sea, Kura River in Tbilisi and southern basin of the Bug River of the Black Sea, respectively). The karyotype of *S. baltica* Witkowski, 1994 from the Bug River in Poland was reported by Boroń (2000). All these studies document invariable  $2n = 50$  and remarkably small karyotype variability among the loaches of this genus. To contribute to the cytotaxonomy of *Sabanejewia*, the present report describes the karyotype and C-banding pattern of *S. bulgarica* from Danube Delta, Romania. Additionally, the comparison of karyotypes of all karyologically studied species of *Sabanejewia* was conducted.

## Materials and methods

The five *Sabanejewia bulgarica* specimens examined (all females) were collected in the Saint George Branch, Danube Delta (July 1997). The loach individuals arrived at the laboratory (Danube Delta Institute, Tulcea) in very bad condition and died subsequently. One female displayed the colour pattern and high body as described in original description by Drensky (1928), while four other individuals displayed other colour patterns (Fig. 1).

Standard procedure for chromosome preparation followed Ráb and Roth (1988) and C-banding technique followed Haaf and Schmid (1984). Chromosomes were clas-



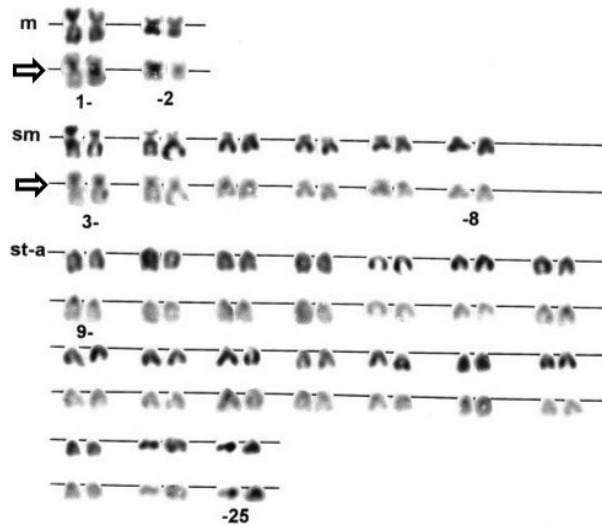
**Figure 1.** Analysed females of *Sabanejewia bulgarica* from the Saint George Branch, Danube Delta  
**A** individual with colour pattern and high body as described in original description by Drensky (1928)  
**B** individuals of *S. bulgarica* with other colour patterns.

sified according to the system of Levan et al. (1964). The analysed individuals are deposited as vouchers in fish collection of Laboratory of Fish Genetics, IAPG, AS CR, Liběchov (Accs. code. SB 7/97).

## Results and discussion

The diploid chromosome number was determined as  $2n = 50$  in all five individuals. The detailed karyotype analysis was carried out on a single specimen with morphological characters corresponding to the original description (Fig. 1, top individual). The karyotype comprised 2 pairs of metacentric (m), 6 pairs of submetacentric (sm) and 17 pairs of subtelocentric (st) to acrocentric (a) chromosomes (Fig. 2). C-banding procedure reveals conspicuous pericentromeric blocks of heterochromatin in most of the chromosome pairs with very prominent blocks in the largest m chromosome and the first sm element.

Our results enable us to compare the karyotype of *Sabanejewia bulgarica* with karyotypes of other species of this genus analysed so far (Table 1). It is evident that the karyotype of *S. bulgarica* shows some apparent similarities with those of congeneric species: i) the same diploid chromosome number  $2n = 50$ , ii) the low number of m chromosomes (two pairs), iii) the numbers of sm chromosomes (six pairs) and st to a chromosomes (17 pairs), iv) likely C-banding pattern correspond to those



**Figure 2.** Karyotype of female *Sabanejewia bulgarica* arranged from conventionally Giemsa-stained (the first row) and sequentially C-banded (the second row, large blocks denoted by arrows) chromosomes. m – metacentric, sm – submetacentric, st – subtelocentric and a – acrocentric chromosomes.

**Table 1.** Diploid chromosome numbers (2n) and karyotype structure of karyologically studied species of genus *Sabanejewia*. Types of chromosomes: m – metacentric, sm – submetacentric, st – subtelocentric, a – acrocentric.

Species	2n	Karyotype characteristics				References
		m	sm	st	a	
<i>S. larvata</i>	50	2	3	11	9	Lodi and Marchioni 1980
<i>S. caspia</i>	50	2	3	11	9	Vasil'ev 1985; Vasil'ev and Vasil'eva 1994
<i>S. kubanica</i>	50	3	7		15	Vasil'eva and Vasil'ev 1988
<i>S. aurata aurata</i>	50	3	6		16	Vasil'ev and Vasil'eva 1994
<i>S. (aurata) balcanica</i>	50	2	6		17	Ráb et al. 1991
<i>S. (aurata) balcanica</i>	50	2	6		17	Vasil'ev and Vasil'eva 1994
<i>S. baltica</i>	50	2	8		15	Boroń 2000
<i>S. bulgarica</i>	50	2	6		17	This study

of *S. balcanica* (Ráb et al. 1991). The comparison also demonstrates some differences in karyotype structure, which, however, need explanation. Lodi and Marchioni (1980) and Vasil'ev (1985) distinguished the categories of st and a chromosome while Vasil'eva and Vasil'ev (1988), Ráb et al. (1991) and Vasil'ev and Vasil'eva (1994, 2019) did not recognize these morphological types of chromosomes as different ones but combined them together. This is especially due to the presence of small acrocentric-like chromosomes with their centromere position ranging gradually from subterminal to nearly terminal which makes difficult the precise assignment of these chromosomes to particular morphological types. The possible interspecific differences in proportion of such st and a chromosomes are in addition masked by the effect of chromosome arms contraction during mitosis due to the effect of timing and dose

of colchicine treatment. Thus, it is difficult to interpret the karyotype descriptions of different authors.

In conclusion, the karyotype of *S. bulgarica* from the Danube Delta is very similar to karyotypes of other *Sabanejewia* species in respect of the chromosome number and morphological types of chromosomes, and is nearly identical with karyotype of *S. balcanica* from the Northern Carpathian range in Slovakia. Further detailed cytotoxicological as well as other genetic surveys of populations of *Sabanejewia* throughout its distribution will answer problems of systematics of Danubian golden loaches.

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## References

- Bănărescu P (1964) Fauna Republicii Populare Romine. Pisces–Osteichthyes. Editura Academiei Republicii Populare Române, București, 962 pp.
- Bănărescu P, Nalbant T, Chelmu S (1972) Revision and geographical variation of *Sabanejewia aurata* in Romania and the origin of *S. bulgarica* and *S. romanica* (Pisces, Cobitidae). *Annotationes Zoologicae et Botanicae* 75: 1–49.
- Boroń A (2000) Cytogenetic characterization of the loaches of the genera *Sabanejewia*, *Misgurnus* and *Barbatula* (Pisces, Cobitidae). *Folia Zoologica* 49(Suppl. 1): 37–44.
- Drensky P (1928) Die Fische der Familie Cobitidae in Bulgarien (nach der Sammlung der Konigl. Natur-Historischen Museums in Sofia). *Bulletin de l'Institut Royal des Sciences Naturelles Sofia* 1: 156–181. [in Bulgarian, German summary]
- Haaf T, Schmid M (1984) An early stage of ZW/ZZ sex chromosome differentiation in *Poecilia sphenops* var. *melanistica* (Poeciliidae, Cyprinodontiformes). *Chromosoma* 89: 37–41. <https://doi.org/10.1007/BF00302348>
- Kottelat M (1997) European freshwater fishes. *Biologia, Bratislava* 52/Supplement 5: 1–271.
- Kottelat M (2012) *Conspectus Cobitidum: an inventory of the loaches of the world (Teleostei: Cypriniformes: Cobiotoidei)*. *Raffles Bulletin of Zoology Supplement* 26: 1–199.
- Kottelat M, Freyhof J (2007) *Handbook of European Freshwater Fishes*. Cornol, Publications Kottelat, CH, 646 pp.
- Křížek P, Mendel J, Fedorčák J, Koščo J (2020) In the foothill zone – *Sabanejewia balcanica* (Karaman 1922), in the lowland zone – *Sabanejewia bulgarica* (Drensky, 1928): Myth or reality? *Ecology and Evolution* 10(14): 7929–7947. <https://doi.org/10.1002/ece3.6529>
- Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201–220. <https://doi.org/10.1111/j.1601-5223.1964.tb01953.x>

- Lodi E, Marchioni V (1980) Chromosome complement of the masked loach *Sabanejewia larvata* (De Fil.) (Pisces, Osteichthyes). *Caryologia* 33: 435–440. <https://doi.org/10.1080/00087114.1980.10796857>
- Nalbant TT (1957) *Cobitis aurata vallahica*, eine neue Unterart des Balkan-Steinpeitzgers (Pisces, Cobitidae). *Senckenbergiana biologica* 38: 209–232.
- Nalbant TT (1994) Studies on loaches (Pisces: Ostariophysi: Cobitidae). I. An evaluation of the valid genera of Cobitinae. *Travaux du Museum National d'Histoire Naturelle „Grigore Antipa“* 34: 375–380.
- Perdices A, Doadrio I (2001) The molecular systematics and biogeography of the European cobitids based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* 19: 468–478. <https://doi.org/10.1006/mpev.2000.0900>
- Pietschman V (1937) Eine neue Lokalform von *Cobitis taenia* L. (Cyprinoidea), *Cobitis taenia tessellatus*. *Anzeiger der Akademie der Wissenschaften in Wien* 74: 29–30.
- Ráb P, Roth P (1988) Cold-blooded vertebrates. In: Balíček P, Forejt J, Rubeš J (Eds) *Methods of Chromosome Analysis*, Czechoslovak Biological Society Publishers, Brno, 115–124. [in Czech]
- Ráb P, Roth P, Vasil'eva ED (1991) Chromosome banding study of the golden loach *Sabanejewia aurata balcanica* from Slovakia (Cobitidae). *Japanese Journal of Ichthyology* 38: 141–146. <https://doi.org/10.1007/BF02905539>
- Šlechtová V, Bohlen J, Perdices A (2008) Molecular phylogeny of the freshwater fish family Cobitidae (Cypriniformes: Teleostei): Delimitation of genera, mitochondrial introgression and evolution of sexual dimorphism. *Molecular Phylogenetics and Evolution* 47: 812–831. <https://doi.org/10.1016/j.ympev.2007.12.018>
- Vasil'ev VP (1985) *Evolutionary Karyology of Fishes*. Nauka Publishers, Moscow, 300 pp. [in Russian]
- Vasil'ev VP, Vasil'eva ED (1994) The karyological diversity in spined loaches from genera *Cobitis* and *Sabanejewia*. Abstract VIII Congress of Societatis Europaea Ichthyologorum “Fishes and their Environment”, Oviedo, Spain, 26 Sept.–2 Oct. 1994, 67 pp.
- Vasil'eva ED, Vasil'ev VP (1988) Studies in intraspecific structure of *Sabanejewia aurata* (Cobitidae) with the description of new subspecies *S. aurata kubanica* subsp. nov. -*Voprosy Ichtiologii* 28: 192–212. [in Russian]
- Vasil'eva ED, Vasil'ev VP (2019) Caspian spined loach *Sabanejewia caspia*: well-known but practically unexplored species of the Cobitidae family: peculiarities of morphology, karyotype, distribution, and postulated phylogenetic links. *Journal of Ichthyology* 59(2): 144–159. <https://doi.org/10.1134/S0032945219020206>
- Vladykov V (1925) Über eine neue *Cobitis*-art aus der Tschechoslowakei: *Cobitis montana* sp. n. *Zoologische Jahrbucher, Abteilung für Systematik, Geographie und Biologie der Tiere* 50: 320–338.

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